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Pre-ovulatory follicle size at induced estrus and post-ovulatory luteal profiles, and pregnancy rate in Murrah buffalo (*Bubalus bubalis*) using estradiol-17β + CIDR protocol

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It is speculated that pre-ovulatory follicle (POF) size is responsible for subsequent development of CL and hence conception in cattle as well as buffalo. However, the study in buffaloes under Indian conditions is still a limitation to improve conception rate. Therefore, there is a need to study intensively the impact of pre-ovulatory follicle size on the subsequent luteal profiles and subsequent conception rate in buffaloes. In fact, it has been proposed that the POF diameter is related to pregnancy outcome and it was found higher following induced ovulation of 14.5 mm follicle than that of 10.3 mm follicles (Perry *et al.* 2005). Moreover, it was observed in a recent study that the diameter of POF in buffaloes has positive impact on plasma estradiol concentration at estrus (Pandey *et al.* 2010). Campanile *et al.* (2005) and Lopes *et al.* (2007) have suggested that throughout the post-ovulation luteal phase, the pregnant buffaloes had higher plasma progesterone concentration than non-pregnant buffaloes. The present study was focused on to determine the relationship between the size of pre-ovulatory follicle and plasma estradiol on the day of estrus as well as subsequent luteal profiles on day 5, 12 and 16 post-ovulation. Also whether a larger pre-ovulatory follicle will generate a large CL which may subsequently lead to increase in progesterone concentration in buffalo. The estradiol + CIDR based protocol was applied for induction of estrus as it was tested in our preliminary study to result in more intense form of estrus in buffalo.

Animals selected were between second to fourth parity, body weight ranging between 400–500 kg and body condition score was 4–5 as per the guidelines of BCS chart.

On day 0 (beginning of the experiment), buffaloes were administered controlled internal drug release (CIDR) device (1.38 g progesterone). Concurrently, buffaloes received 1.5 mg of estradiol-17β (Sigma) in 2 ml oil (canola oil, i.m). On day 9, CIDR was removed and each buffalo was administered $PGF_2\alpha$ analogue (Cloprostenol sodium, 500 µg, i.m.; Vetmate, Vetcare, Provimi). On day 11, buffaloes were administered GnRH analogue (Buserelin acetate, 20 µg, i.m.; Receptal, Intervet). All the buffaloes were inseminated on day 11 (12 h) and day 12 (24 h).

Transrectal ultrasonography (Linear B mode, Agroscan, 7.5 MHz) was carried out on day 0 (day of ovulation), and day 5, 12 and 16 post-ovulation for monitoring the ovarian status. Ovaries were systematically examined for the diameter of visible CL. Blood samples were collected on day 0 (day of ovulation), and day 5, 12 and 16 post-ovulation in heparinized vials. Plasma was separated immediately after blood collection and frozen at –20°C until analysis.

Plasma progesterone was estimated by liquid phase Radio-immunisation (RIA) procedure using polyclonal progesterone antiserum raised in the RIA laboratory (Ghuman *et al.* 2009) located in the Department of Veterinary Gynaecology and Obstetrics, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana. The sensitivity of the assay is 0.1 ng/ml for progesterone. The mean intra- and inter-assay coefficient of variance were 6.2 and 9.5%, respectively.

Plasma estradiol-17β (E_2) was estimated using a commercially available Direct Immuno-enzymatic assay kit (Monobind Inc. USA, Accubind estradiol ELISA microwells). Absorbance of each well was taken at 450 nm within 30 min. Standard curve was elaborated with the 4 parameters curve fitting system and the estradiol concentration in samples was calculated.

The diameters of POF and CL were calculated on the bases of average of longest and smallest axis diameters of individual follicles and corpora lutea measured by ultrasonography.

The relationship between the diameter of pre-ovulatory follicle and size of CL and plasma progesterone on day 5, 12, and 16 was analyzed using correlation coefficient. Correlation was also determined between size of POF and plasma estradiol on the day of estrus. Statistical analysis was performed using the SPSS (16.0) system for windows.

There was positive correlation between plasma progesterone level on day 5 and day 12 post-ovulation and

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the diameter of POF on the day of estrus (d5 progesterone: r=0.21, P>0.05; d12 progesterone: r=0.14, P<0.05) in pregnant as well as non-pregnant animals. Diameter of CL on day 5 and 12 post-ovulation was positively correlated with diameter of POF (Table 1) on the day of estrus (d5 CL and POF: r=0.67, P<0.05; d12 CL and POF: r=0.56, P<0.05) in pregnant as well as non-pregnant animals. However, correlation between plasma progesterone level and diameter of CL on day 12 post-ovulation was positive only in pregnant animals. Correlation between POF and plasma estradiol was 0.14 (POF vs plasma estradiol, r=0.14, P<0.05).

The attempts to study the relationship between the size of pre-ovulatory follicle and post-ovulatory luteal profiles and conception in buffaloes needs interventions as there is still variability in results obtained so far. Previous studies reported that both CL diameter and plasma progesterone were positively correlated with POF diameter (Robinson *et al.* 2005, Mann 2009 and Pandey *et al.* 2010). In contrast, no correlation between the diameter of POF and subsequent CL diameter as well as with progesterone level on day 7 post-estrus had been reported (Lynch *et al.* 2010). Although much results are available in literature for cattle but the variability of results warns further intensive studies particularly in case of buffaloes. From the findings of the present study, it could be suggested that a large pre-ovulatory follicle generates a large CL which subsequently leads to high progesterone and increases the chances of pregnancy. Similar hypothesis had been proposed by other workers (Binelli *et al.* 2001, Lopes *et al.* 2007, Busch *et al.* 2008, Pfeifer *et al.* 2009 and Pandey *et al.* 2010).

The results also revealed that plasma progesterone and diameter of CL were positively correlated on day 5 as well as on day 12 post-ovulation in pregnant animals (P_4 vs CL, day 5, r=0.22; day 12, r=0.44) but was weekly correlated on day 5 and no correlation on day 12 post-ovulation in non-pregnant counterparts. Pregnant animals in the present study, as confirmed through ultrasonography, conceived at first service. This was in agreement with previous studies in cows and buffaloes which strongly support the positive correlation between progesterone and CL diameter till day 8 post-ovulation as well as plasma progesterone and pregnancy (Campanile *et al.* 2005, Kavani *et al.* 2005 and Grimard *et al.* 2006). Also, the present study reveals that the correlation between plasma progesterone and CL diameter is positive in early as well as mid-luteal phase in pregnant animals. The positive correlation between plasma progesterone and CL diameter indicates that larger CL results in increased plasma progesterone level and increases the chances of pregnancy in buffalo. Also increase in CL diameter (19.4% from day 5 to day 12 post ovulation), as observed through ultrasonography in the present study might be associated with the increase in progesterone which may favour a successful conception particularly at critical stage of embryonic development. The absence of correlation between plasma progesterone and CL diameter in non-pregnant animals might be related to the less functional CL developed in post ovulatory luteal phase. Since the present study was conducted on anestrus animals using estradiol-based synchronization regimen, much literature in this regard is not available.

Table 1. Coefficient of correlation (r) between post ovulatory luteal profiles and diameter of pre-ovulatory follicle of pregnant and non-pregnant animals within the group

Parameter	Pregnant (n=12)	Non Pregnant (n=18)
CL and POF (Day 5)	0.67	0.22
P_4 and POF (Day 5)	0.21	0.70
CL and POF (Day 12)	0.56	0.32
P_4 and POF(Day 12)	0.14	0.07

CL, Corpus luteum; POF, Pre-ovulatory follicle; P4, Plasma Progesterone.

In the present study, diameter of pre-ovulatory follicle increased significantly (P<0.05) 48 h after CIDR withdrawal. Also, a concomitant rise in estradiol concentration observed on the day of estrus, suggests that there is a positive correlation between between the size of POF and plasma estradiol in buffaloes. Further, compared to non-pregnant buffaloes, pregnant buffaloes bearing larger POF diameter (13.8 mm vs 12.4 mm) showed higher plasma estradiol level on the day of estrus (0.64 vs 0.44 ng/ml. This was in agreement with previous reports which have suggested positive correlation between estradiol synthesis by the POF and the subsequent pregnancy rates in cattle (Nosier 2003, Lopes *et al.* 2007 and Perry *et al.* 2007). The higher estadiol level in pregnant animals at the time of estrus, (n=6, P<0.05) in the present study may be associated with proper LH surge and fixed time ovulation. This is supported by a study in *Bos indicus* by Maquivar *et al.* (2007) where they suggested that estradiol induced LH surge might be a good marker for timed ovulation. Since our study revealed higher estradiol levels in pregnant animals at the time of estrus (n=6, P<0.05), hence the higher estadiol level might be associated with proper LH surge and fixed time ovulation which later might result in higher pregnancy rates in buffalo.

Thus as a conclusion of the present study, both the plasma progesterone level as well as size of CL were positively correlated with diameter of POF on the day of estrus. The larger the size of pre-ovulatory follicle, the higher the level of estradiol on the day of estrus. Also, larger pre-ovulatory follicle generates a large CL which subsequently leads to high progesterone and ultimately can improve pregnancy in buffalo.

SUMMARY

The present study was designed with the objective to assess the relationship between pre-ovulatory follicle (POF) size and post-ovulatory luteal profiles in buffalo. The study was conducted on 30 Murrah buffaloes maintained at two private dairy farms. The buffaloes had the history of anestrum and were induced to estrus following synchronization with estradiol-based protocol. On day 0

(beginning of experiment), buffaloes were administered controlled internal drug release (CIDR) device (1.38 g P_4) and concurrently received 1.5 mg estradiol-17b (i.m.). On day 9, CIDR was removed and a prostaglandin (PG) F_{2a} analogue (500 µg, Cloprostenol sodium, i.m) was administered. On day 11, buffaloes received GnRH analogue (20 µg, Bucerelin acetate, i.m.) and were inseminated. Trans-rectal ovarian ultrasonography and blood sampling was carried out on day 0 (day of ovulation), and day 5 and 12 post-ovulation for monitoring the ovarian status. Correlation coefficient was determined between the size of POF and luteal profiles, size of corpus luteum (CL) and progesterone concentration on day 5 and 12 post-ovulation as well as size of POF and plasma estradiol on the day of estrus. In conclusion, in Murrah buffalo, there was positive correlation between plasma progesterone level on day 5 and day 12 post-ovulation and the diameter of POF on the day of estrus. Also the correlation was positive between size of CL and progesterone level as well as between size of POF and plasma estradiol level on the day of estrus.

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